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OVARY STRUCTURE AND OOCYTE SIZE IN RELATION TO FEMALE SIZE AND AGE IN THE BROOD PARASITIC WASP *STIZOIDES RENICINCTUS* (SAY) (HYMENOPTERA: CRABRONIDAE)

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Abstract.—*Stizoides renicinctus* (Say) is a brood parasitic bembicine wasp that lays eggs in nests of nest-provisioning species of *Palmodes* and *Prionyx*, where its larvae feed on the host's provisions. Published comparative evidence based on a limited number of dissections in each species suggests that females of *Stizoides* and other brood parasitic Bembicinae have more have more ovarioles (8 vs. 6) and carry a greater number of relatively small mature oocytes than do nest-provisioning bembicines. To explore this further, we studied of a larger sample of *S. renicinctus*. Most females actually had 10 ovarioles, two more than any previous record reported for apoid wasps. Females also carried more mature oocytes (up to 6) and relatively smaller mature oocytes than their nest-provisioning relatives among the Bembicinae. In addition, we found a positive correlation of female *S. renicinctus* body size with the number and size of oocytes carried. And older females, as judged by their greater wing wear, tended to carry relatively fewer and smaller oocytes. Thus, there may be both size- and age-related constraints on reproduction in this species.

Key Words: Crabronidae, Bembicinae, *Stizoides renicinctus*, brood parasite, ovariole number, oocyte number, body size, age

Besides providing offspring with a nest and prey, solitary nest-provisioning wasps also lay relatively large eggs. Because of this and because of space limitations in their abdomens, female nest-provisioners tend to carry relatively few mature eggs at any one time, usually 0–2, even though they have six ovarioles (Iwata 1955, 1960; Itô 1978, O'Neill 1985, 2001; Ohl and Linde 2003). The smaller number of mature eggs carried may not limit the rate of offspring production, because nest provisioners often spend one or more days preparing and provisioning a single brood cell, perhaps allowing time for the next largest oocyte to mature. The situation may be different, however, for related parasitoid and brood parasitic species that have a potentially higher rate of egg-laying as they move from host to host without being constrained by the need to construct and provision a nest. The hypothesis that parasitoid and brood parasitic wasps carry a greater number of mature eggs that are smaller than those of similar-sized nest provisioners is generally supported by comparative evidence (Itô 1978, O'Neill 2001, Ohl and Linde 2003).

Among apoid wasps, all obligate brood parasites are in the subfamily

Bembicinae of the Crabronidae. Recently, Ohl and Linde (2003) reported on the number of ovarioles and mature oocytes of 68 species of apoid wasps, and reviewed earlier studies by Iwata (1955, 1960). With one exception, the mean number of mature oocytes in the 16 species of nest-provisioning Bembicinae ranged from 0 to 2, and all 16 species had three ovarioles in each of their two ovaries. In contrast, all nine species of brood parasitic Bembicinae had four ovarioles per ovary, and the 12 individuals examined in nine species carried 2–5 (mean = 4.2) mature oocytes. One of the North American species examined was *Stizoides renicinctus* (Say), a brood parasite of wasps of the genera *Palmodes* Kohl and *Prionyx* Vander Linden (Sphecidae) (Evans 1966). The single female *S. renicinctus* dissected by Ohl and Linde had five mature oocytes distributed among eight ovarioles. But, there is relatively little information available on intraspecific variation in ovariole number and oocyte size in apoid wasps. Because of this, Ohl and Linde (2003) note that the hypothesis that brood parasitic apoid wasps carry relatively smaller eggs than their nest provisioning relatives needs to be tested with larger data sets. Recently, we had the opportunity to examine a larger sample of female *S. renicinctus*, with the aim of documenting intraspecific variation in oocyte size and number, as well as its relationship to female size and age.

Materials and Methods

We collected *S. renicinctus* at two locations in Montana: 1) 14 km south of Three Forks, Gallatin, Co. and 2) 4 km southeast of Bridger, Carbon Co. at the USDA-NRCS Bridger Plant Materials Center. All specimens of *S. renicinctus* were preserved in Kahle's solution soon after capture. We recorded each female's maximum head width (to nearest 0.05 mm) and thorax width (to nearest 0.04 mm, measured as the "distance between the outer extremities of the tegulae"; Iwata and Sakagami 1966). We then dissected females in ethanol under a stereomicroscope fitted with an ocular micrometer, and recorded the number, length (L), and width (W) of all mature oocytes (to the nearest 0.4 mm), and the total number of ovarioles and visible oocytes. An oocyte was scored as mature if it was pale yellow and generally sausage-shaped, and lacked associated nurse cells. The volume (V) of mature oocytes was estimated as: $V = (L - 2r)\pi r^2 + (4\pi r^3/3)$, where r (radius) = L/2 (O'Neill 1985). For each female, we also calculated an "egg index," the ratio of the length of the largest egg carried by a female to thorax width, which facilitates comparisons of relative egg size among females of different sizes, including females of different species (Iwata and Sakagami 1966).

Results and Discussion

At Three Forks, we collected females from two diffuse sleeping clusters, each containing two female *S. renicinctus* and two *Prionyx* sp.; the two clusters were about 60–70 cm high on yellow sweetclover (*Melilotus officinalis* L.). At Bridger, we found eight sleeping clusters on *Kochia scoparia* (L.) Schrad. protruding above cultivated rows of *Dalea candida* Michx. ex Willd. Two clusters collected together from Bridger contained a total of 52 *S. renicinctus* (7 males, 45 females), along with 1 male *Prionyx canadensis* Provancher (Sphecidae), 1 male *Stenodynerus* sp., 5 female *Euodynerus* sp., and one male *Agapostemon angelica* Cockerell. Another single cluster contained 11 *S. renicinctus* (10 males, 1 female), 3 *P. canadensis* (2 males, 1 female), 1 male *Sphex lucae* Lepeletier, 1 *Ammophila* sp., and 6 male *Euodynerus* sp.. The *S. renicinctus* perched close to one another near the plant's central stem, and grasped either the main stem, leaflets just off of the main stem, or each other. At both sites, clusters were seen during late morning or early afternoon on partly cloudy or overcast days. Mixed-species sleeping clusters that include *Stizoides*, along with potential hosts, also have been reported in Rau (1938) and (Ohl 1999).

The ovaries of *S. renicinctus* had 8 (N = 12), 9 (N = 1) or 10 (N = 18) ovarioles each; females with 8 vs. 10 ovarioles did not differ in head width (Mann-Whitney test, $P = 0.64$). As far as we know, this is the first report of an apoid wasp with more than 8 ovarioles (Iwata 1955, 1960; Ohl and Linde 2003). Among aculeate wasps, ovariole numbers exceeding six have been reported only for parasitoids of the families Mutillidae (up to 10) and Chrysididae (usually 8–10), for eusocial Vespidae (up to 22), and for brood parasitic Bembicinae of the tribe Nyssonini (6–8) (Ohl and Linde 2003). Intraspecific variability in ovariole number has also been reported in *Mutilla europaea* L. (8–10), *Chrysis ignita* L. (8–10), and *Vespa* spp., as well as in the brood parasitic bee *Nomada japonica* Smith (8–11) (Iwata 1955).

Ovaries of *S. renicinctus* carried 18–38 oocytes (visible at 40× magnification) in all stages of development (mean ± SE = 27.0 ± 1.0) and 1–6 mature oocytes (mean ± SE = 3.6 ± 0.2); we never found more than one mature oocyte per ovariole. The average number of mature oocytes per ovariole (0.40) was within the range of values reported for other bembicine brood parasites (0.25–0.84), and higher than most values reported for bembicine nest provisioners, which generally range from 0.17–0.33 (Ohl and Linde 2003). The mean length of mature oocytes was 2.58 ± 0.04 mm (range: 2.1–3.1 mm), whereas their mean volume was 0.72 ± 0.03 mm^3 (range: 0.38–1.15 mm^3).

Iwata and Sakagami (1966), Itô (1978), and Ohl and Linde (2003) compared relative egg sizes among parasitoid, brood parasitic, and nest provisioning solitary wasps with different body sizes using the "egg index." Egg indices have been published for a number of solitary aculeate wasps, but 58 of the 90 published values for crabronid wasps are for species for which just a single female was dissected, and another 23 were derived from samples of 2–3 females (Ohl and Linde 2003). The mean egg index for 30 *S. renicinctus* females in our sample was 0.77 ± 0.02 (range: 0.65–0.96). The fact that the egg index was quite variable and that it varied inversely with body size (head width-egg index correlation, Spearmann correlation, $r = -0.86$, $P < 0.001$), gives further indication that larger data sets are needed to characterize relative egg size.

The mean egg index for *S. renicinctus* is similar to the mean (0.74) for the other seven species of brood parasitic Bembicinae in Ohl and Linde (2003). In contrast, the seven species of nest-provisioning crabronids on Table 1 (the only species with sample sizes greater than 10) have egg index values 9–22% higher than that of *S. renicinctus*; the 22% value being that for *Bembecinus quinequespinosus* (Say), a member of the same tribe (Stizini) as *S. renicinctus*. The range in the sizes of the largest mature oocytes carried by *S. renicinctus* is about the same as that for *B. quinquespinosus* and *Philanthus pulcher* Dalla Torre, although both are smaller than *S. renicinctus*, much smaller in the case of *P. pulcher*. Unlike mature oocytes in ovaries of *Philanthus* (O'Neill 1985), those of *S. renicinctus* appeared much less crowded and deformed by other organs and tissues in the abdomen. Brood parasitic solitary bees also have relatively smaller mature oocytes compared to similar-sized nest provisioners (Alexander and Rozen 1987).

Despite the differences documented above, *S. renicinctus* is similar to its nest-provisioning relatives in that head

Table 1. Egg indices for species of Crabronidae for which more than 10 females have been examined.

Species	N	Egg Index	Source
Bembicinae			
Bembecinus quinquespinosus (Say)	30	0.94	O'Neill 1985[1]
Bembix americana F.	30	0.84	O'Neill 1985[1]
Stizoides renicinctus (Say)	31	0.77	present study
Philanthinae			
Cerceris graphica Smith	32	0.84	Ohl and Linde 2003
Cerceris morata Cresson	13	0.95	Ohl and Linde 2003
Philanthus bicinctus (Mickel)	21	0.90	O'Neill 1985[1]
Philanthus pulcher Dalla Torre	40	0.92	O'Neill 1985[1]
Philanthus zebratus Cresson	41	0.86	O'Neill 1985[1]

[1] Egg index calculated from egg length data in O'Neill (1985) and our unpublished measurements of thorax width.

width was correlated, intraspecifically, with 1) the number of visible oocytes ($r = 0.84$, $P < 0.001$), 2) the number of mature oocytes ($r = 0.59$, $P < 0.001$), 3) the number of mature oocytes per ovariole ($r = 0.51$, $P = 0.004$) and 4) the length ($r = 0.65$, $P < 0.001$) and volume ($r = 0.66$, $P < 0.001$) of each female's largest oocyte. Thus, larger female *S. renicinctus* may be better able to exploit a series of host nests in rapid succession, and to lay larger, more yolk-rich eggs than can smaller conspecifics. Among apoid wasps, higher fecundity and smaller relative egg size has evolved secondarily, not only among the brood parasitic Bembicinae, but among the few parasitoid sphecids (e.g., *Larra* spp., *Chlorion lobatum* F.) (O'Neill 2001, Ohl and Linde 2003). In addition, some ground-nesters that make a series of simple single-celled nests over short time intervals may have evolved the ability to reduce the time between successive ovipositions by carrying greater numbers of smaller mature eggs. For example, each female of *Podalonia valida* (Cresson), which carry as many as seven mature oocytes, provisions a series of shallow single-celled nests in close proximity to one another, and does so more rapidly than relatives that dig more complex nests provisioned with multiple prey per cell (O'Neill and Evans 1999).

We also found evidence of age-related constraints on the quantity and size of eggs. The age of females, judged by the amount of wing wear, was correlated with the number, size, and condition of oocytes they carried. Thirteen females had no visible wing wear, so were likely relatively young, whereas 17 displayed varying degrees of tattering and tearing at wing edges; females in the two samples did not differ in size (Mann-Whitney test, $P = 0.16$). Compared with females with at least some wing wear, those having no wing wear carried a greater number of mature oocytes per ovariole (mean $= 0.47 \pm 0.05$ vs. 0.34 ± 0.02; Mann-Whitney test, $P = 0.02$). The largest oocyte carried by younger females also had a greater volume than that carried by older females (mean $= 0.80 \pm 0.05$ vs. 0.66 ± 0.03 mm^3; Mann-Whitney test, $P = 0.04$). Eleven (61%) of the females with at least some wing wear had one terminal oocyte that was apparently in the process of being resorbed (i.e., it was often as long as normal, mature terminal oocytes, but was very slender and often deformed). In contrast, just one (8%) of females with no wing wear had such an oocyte ($\chi^2 = 7.00$, $P =$

0.008). Thus, there may be both size- and age-related constraints on reproduction of *S. renicinctus* females.

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